ORIGINAL ARTICLE

SERUM HOMOCYSTEINE AS A RISK FACTOR FOR CORONARY HEART DISEASE

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Background: Homocysteine (Hcy) is an intermediate formed during the catabolism of sulphur containing essential amino acid, methionine and Less than one percent of tHcy is found as the free form. Development of atherosclerotic changes and thrombo-embolism are common features in patients with homocysteinuria. This study was conducted to assess the relationship of Hcy and coronary heart disease (CHD) in our population. **Methods:** The cross-sectional analytical study was carried out at the Department of Biochemistry, Hazara University Mansehra and Ayub Medical College, Abbottabad. A total of 80 subjects were included in this study and were divided into 2 groups. Cases Group consisted of 40 patients who had confirmed Myocardial Infarction (MI) coming for routine follow-up (first re-visit) after the acute attack. Control Group consisted of 40 matching healthy individuals. Demographic data including age, gender, dietary habits, height and weight as documented in preformed proforma. Blood pressure was taken in sitting posture. Serum total Hcy were measured. Data was entered into computer using SPSS 16.0 for analysis. **Results:** The mean age of the cases was 59.68±8.06 (30–70) years and that of the controls was 58.93±6.93 (48–76) years. The average BMI of cases was 27.70±3.61 Kg/m^2 and of the controls was 25.66±2.98 Kg/m^2. This increase of BMI from controls to cases was statistically significant (p<0.050). The mean systolic BP of the cases was 153.88±11.90 mmHg in comparison with 142.62±11.65 mmHg for the controls. This difference was statistically significant (p<0.001). Mean tHcy level of the cases was 17.15±4.45 µmol/l while that of controls was 12.20±2.53 µmol/l. There is a statistically significant difference between cases and controls with respect to Hcy levels (p<0.001). **Conclusion:** Plasma tHcy level has a powerful predictor value of CHD and routine screening for elevated Hcy concentrations is advisable especially for individuals who manifest atherothrombotic disease without their traditional risk factors.

Keywords: Hyperhomocysteinemia (HHcy), coronary heart disease (CHD)

INTRODUCTION

Homocysteine (Hcy) is an intermediate formed during the catabolism of sulphur containing essential amino acid, methionine.[1,2] It is found either as free Hcy, cysteine-Hcy mixed disulfide or protein bound Hcy. The one that is bound with protein in plasma reflects total plasma Hcy (tHcy).[3] In the mid 1960 Kilmer Mc Cully, treated an 8 years old girl with a rare inherited metabolic condition (homocystinuria) that causes Hcy levels to rise. The autopsy of this patient showed massive generalised atherosclerosis. Mc Cully speculated the cause of atherosclerosis to be high Hcy levels. He wondered if the same was true for atherosclerosis in adults. Mc Cully hypothesised that even slightly elevated Hcy levels could cause atherosclerosis in older individuals but his hypothesis failed to get acceptance. Recent studies[4] are now confirming Mc Cully's work.

The development of atherosclerotic changes and thrombo-embolism are common features in patients with homocysteinuria (Heritable defects of different enzymes involved in methionine catabolism). Hence a positive correlation between Hcy and atherosclerosis was postulated.[1] Individuals with elevated levels of Hcy tend to have higher incidence of cardiovascular disease. Hyperhomocysteinemia (HHcy) is an independent risk factor for atherosclerotic diseases, including ischemic heart disease, stroke and peripheral vascular disease.[4,5]

Although the mechanisms responsible for the endothelial damage and how this leads to atherosclerosis and thrombosis are presently unknown. Hcy may cause vascular events which may contribute to heart disease by the epithelial cell injury, affect coagulation proteins and enhance pro-coagulant activity, alter platelet function, modify LDL and may stimulate smooth muscle cell proliferation.[6,7]

Prevalence of CHD risk factors is known to be high in Pakistan.[8] High serum cholesterol levels are an important risk factor for CHD, but most patients with MI have normal cholesterol levels. There is a need to recognise Hcy as a potential risk factor in local population with the help of local evidence.

MATERIAL AND METHODS

The cross-sectional analytical study was carried out at the Department of Biochemistry, Hazara University Mansehra, and Ayub Medical College, Abbottabad. A total of 80 subjects were included in this study by convenience (non-probability) sampling technique. They were divided into 2 groups: Cases,

consisting of 40 confirmed patients of confirmed Myocardial Infarction (MI) coming for routine follow-up (first visit) in the Cardiology OPD after the acute attack; and Controls comprising of 40 age and gender matched healthy individuals.

Patients with known history of liver, kidneys or taking any of the known drugs that affect the liver functions such as isoniazid, phenytoin, chlorpromazine etc. were excluded from the study. The subjects taking folic acid therapy or multivitamins were also excluded from the study.

Informed consent was taken. Demographic data including age, gender, dietary habits, height and weight as documented in preformed proforma. Blood pressure was taken in sitting posture. Ten ml blood was taken from each subject after an overnight fast of 10–12 hours. Samples were centrifuged in a bench top centrifuge at 4,000 rpm for 10 minutes to get serum. Total Hcy were measured. Data was analysed using SPSS-16.

RESULTS

A total of 80 subjects were investigated. Out of these 40 were Cases, and the rest (40) were Controls. The mean age of the cases was 59.68±8.06 (30–70) years and that of the controls was 58.93±6.93 (48–76) years (Table-1). Twenty-three (57.5%) cases and 22 (55%) controls were males whereas 17 (42.5%) cases and 18 (45%) controls were females.

Table-1: Subjects by age groups

	Cases		Controls	
Age Groups (years)	**Number**	**Percent**	**Number**	**Percent**
30–39	1	2.5	0	0
40–49	1	2.5	3	7.5
50–59	16	40.0	21	52.5
60–69	19	47.5	13	32.5
70 and Above	3	7.5	3	7.5
Total	**40**	**100.0**	**40**	**100.0**

Twenty-four (60%) cases and 25 (62.5%) of the controls were on mixed diet. Nine (22.5%) cases and 8 (20%) controls were predominantly on meat diet, while 7 (17.5%) cases and 7 (17.5%) controls were predominantly on vegetable diet. The average weight of the cases was 75.80±12.37 Kg while that of the controls was 58.5±11.73 Kg. Mean height of the cases was 162.62±6.97 Cm and of controls was 163.75±8.51 Cm. The average BMI of cases was 27.70±3.61 Kg/m^2 and of the controls was 25.66±2.98 Kg/m^2. This increase of BMI from controls to cases was statistically significant (p<0.050) (Table-2).

Table-2: BMI of the subjects

Groups	**Height (Cm)**	**Weight (Kg)**	**BMI (Kg/m^2)**
Cases (n=40)	162.62±6.98	75.8±12.38	27.7±3.62
Controls (n=40)	163.75±8.51	58.5±11.73	25.66±2.98
p	>0.05	<0.05	<0.05

The mean systolic BP of the cases was 153.88±11.90 mmHg in comparison with 142.62±11.65 mmHg for the controls. This difference was statistically significant (p<0.001). The mean diastolic BP of the cases was 95.75±9.71 mmHg in comparison with 90.25±9.67 mmHg in the controls (p<0.050). Mean tHcy level of the cases was 17.15±4.45 µmol/l while that of controls was 12.20±2.53 µmol/l (p<0.001). Hcy levels by age groups are tabulated in Table-3.

Table-3: Hcy levels by age groups

	Cases			Control		
Age (Yr)	**No.**	**Mean**	**SD**	**No.**	**Mean**	**SD**
30–39	1	19.200	.			
40–49	1	16.000	.	3	11.367	4.3822
50–59	16	16.500	2.6415	21	12.219	1.8891
60–69	19	17.074	5.3845	13	12.031	3.1420
≥70	3	20.867	6.7419	3	13.667	2.5166
Total	40	17.155	4.4471	40	12.202	2.5310

Among males the mean Hcy levels in cases was 16.9±4.26 µmol/l and in controls was 12.12±2.58 µmol/l, whereas among females the mean Hcy levels in cases was 17.48±4.8 µmol/l and in controls was 12.3±2.5 µmol/l (Table-4).

Table-4: Hcy levels by gender

	Cases			Controls		
Gender	**No.**	**Mean**	**SD**	**No.**	**Mean**	**SD**
Male	23	16.909	4.2677	22	12.123	2.5858
Female	17	17.488	4.7913	18	12.300	2.5333
Total	40	17.155	4.4471	40	12.202	2.5310

DISCUSSION

Atherosclerotic CVD accounts for half of all the premature deaths in men, in the developed as well as in the developing countries.[9] Studies in the last decade have indicated that HHcy is an independent risk factor in the premature development of vascular disease.[10,11] The principal observation of our study was that the mean of serum tHcy levels found in the CHD cases was significantly higher compared to the age and gender matched healthy controls. This finding is in agreement with studies in the West as well as in Pakistan.[12,13]

In a meta-analysis for articles published from Jan 1966 to Jan 1999, relevant studies were identified by systematic research of the literature and data from 30 prospective or retrospective studies was included. It involved a total of 5,073 ischemic heart disease events and 1,113 stroke events. This meta-analysis suggested that elevated Hcy is at the most a modest independent predictor of IHD and stroke risk in healthy population.[14] In another meta-analysis of 27 case control studies Boushey *et al* found that an increase of 5 µmol/l in basal tHcy level was associated with a 60% increase in the odds of CAD among men and an 80% increase in the odds of CAD among women based on which an increase of 5 µmol/l of serum tHcy was estimated to

increase the risk of CAD by as much as 20 mg/dl increase in cholesterol concentration.[15]

The present study also showed that in CHD cases Hcy levels were only moderately elevated. The range of serum tHcy was observed as 8.8–28.6 µmol/l. This finding is also inline with recent observation. This indicates that moderately elevated Hcy level is a risk factor for CHD. None of the cases had Hcy levels more than 100 µmol/l, i.e., severe HHcy. The concentration Hcy at which the risk begins to increase is not clear but a string association between elevated Hcy levels and CHD is reported.[16]

In the present study it was interesting to find that the upper limit of Hcy in the healthy controls of the study was higher than the upper limit of the healthy population in the West (17.9 vs 15 µmol/l). This is because prevalence of vitamin B_{12} and folate deficiency is higher in our part of the world. Our results are in accordance with the other studies which state a high prevalence of HHcy in the Indian population. This may be due to increased prevalence of under-nutritional state and parasitic infestation which is quite common in our part of the world.[17,18] Young Asian Indian men settled in the US and Europe have also been reported to exhibit HHcy which may be due to difference in genetic constitution or difference in cooking and dietary habits.[19]

Bozkaya *et al* found the mean Hcy level 12.18±0.65 µmol/l (Mean±SEM) in the 50 Turkish CHD patients while 3.73±0.43 µmol/l in their matched healthy controls this low level of Hcy can be due to the common usage of multi-vitamin tablets. In addition, fruits and vegetables which are important sources of folic acid and pyridoxine, are produced and consumed in large quantities in Turkey. There is a high intake of plant origin food rather than animal protein. This fact explains the lower mean tHcy levels in the Turkish population.[20]

Elevated Hcy levels were found in 50% of the CHD cases in the present study compared to 40% patients of CHD having elevated Hcy levels in the West.[21] This difference may be due to concurrent covert vitamin and nutritional deficiencies in this part of the world. The difference may be due to small sample size of the study.

Since the mean age of the cases in our study was 59.68±8.068 years, it is unlikely that elevated Hcy in them was due to genetic disorder. Moreover severe HHcy is usually caused by genetically determined enzyme deficiencies that usually manifest as life threatening thrombo-embolic events in early adult life.[22] It has also been observed that all HHcy female cases were in the postmenopausal age. This is in agreement with the recent studies which have found that postmenopausal women with elevated Hcy levels have a higher incidence of CHD.[23]

Several studies have linked serum Hcy level to blood pressure specially systolic BP. Mechanism that could explain the relationship between Hcy and BP include increased arterial stiffness, endothelial dysfunction and low folate status because it is observed that Hcy lowering treatment with folic acid is followed by decrease in BP.[24] In the present study, the correlation between tHcy levels and BP was established which was not significant. This may be attributed to the fact that both the cases and controls belonged to older age groups and many of them were hypertensive patients on antihypertensive therapy.

CONCLUSION

Routine screening for elevated Hcy concentrations is not yet recommended. Screening may be advisable for individuals who manifest atherothrombotic disease without their traditional risk factors or who have a family history of premature CHD.

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